

CLAIMS

1. A purified preparation of pluripotent non-embryonic stem cells, which
 - (i) is capable of proliferating in an *in vitro* culture for more than one year;
 - (ii) maintains a karyotype in which the cells are euploid and are not altered through culture;
 - (iii) maintains the potential to differentiate into cell types derived from the endoderm, mesoderm and ectoderm lineages throughout the culture, and
 - (iv) is inhibited from differentiation when cultured on fibroblast feeder layers.
2. The pluripotent non-embryonic stem cells of claim 1, wherein said cells are negative for expression of the SSEA-1 marker.
3. The pluripotent non-embryonic stem cells of claim 1, wherein said cells express elevated alkaline phosphatase activity.
4. The pluripotent non-embryonic stem cells of claim 1, wherein said cells are positive for expression of the TRA-1-81 marker and the TRA-1-60 marker.
5. The pluripotent non-embryonic stem cells of claim 1, wherein said cells are positive for expression of the CCA-3 and CCA-4 Markers.
6. The pluripotent non-embryonic stem cells of claim 1, wherein said cells differentiate into cells derived from mesoderm, endoderm and ectoderm germ layers when the cells are injected into a SCID mouse.
7. The pluripotent non-embryonic stem cells of claim 1, wherein said cells are human.
8. The pluripotent non-embryonic stem cells of claim 1, wherein said cells are non-human animal cells selected from the group consisting of dog, cat, mouse, rat, cow, pig, sheep, goat, horse, buffalo, llama, ferret, guinea pig and rabbit.
9. The pluripotent non-embryonic stem cells of claim 1, wherein the nuclear DNA has been genetically modified.

10. A purified preparation of pluripotent non-embryonic stem cells, which
(i) is capable of proliferating in an *in vitro* culture for an indefinite period;
(ii) maintains a karyotype in which the cells are euploid and are not altered
5 through culture; and
(iii) maintains the potential to differentiate into cells types derived from the
endoderm, mesoderm and ectoderm lineages throughout the culture.

11. The pluripotent non-embryonic stem cells of claim 10, wherein said cells are
10 negative for expression of the SSEA-1 marker.

12. The pluripotent non-embryonic stem cells of claim 10, wherein said cells express
elevated alkaline phosphatase activity.

13. The pluripotent non-embryonic stem cells of claim 10, wherein said cells are
5 positive for expression of the TRA-1-81 marker and the TRA-1-60 marker.

14. The pluripotent non-embryonic stem cells of claim 10, wherein said cells are
positive for expression of the CCA-3 and CCA-4 Markers.

15. The pluripotent non-embryonic stem cells of claim 10, wherein said cells
differentiate into cells derived from mesoderm, endoderm and ectoderm germ layers when the
cells are injected into a SCID mouse.

16. The pluripotent non-embryonic stem cells of claim 10, wherein said cells are
25 human.

17. The pluripotent non-embryonic stem cells of claim 10, wherein said cells are non-
human animal cells selected from the group consisting of dog, cat, mouse, rat, cow, pig, sheep,
30 goat, horse, buffalo, llama, ferret, guinea pig and rabbit.

18. The pluripotent non-embryonic stem cells of claim 10, wherein the nuclear DNA
has been genetically modified.

19. A stem cell which does not originate from a fertilized egg, but which originates from the combination of a somatic cell nucleus and an enucleated ooplastoid.

5 20. The stem cells of claim 19, wherein said cells are negative for expression of the SSEA-1 marker.

21. The stem cells of claim 19, wherein said cells express elevated alkaline phosphatase activity.

10 22. The stem cells of claim 19, wherein said cells are positive for expression of the TRA-1-81 marker and the TRA-1-60 marker.

15 23. The stem cells of claim 19, wherein said cells are positive for expression of the CCA-3 and CCA-4 Markers.

24. The stem cells of claim 19, wherein said cells differentiate into cells derived from mesoderm, endoderm and ectoderm germ layers when the cells are injected into a SCID mouse.

20 25. The stem cells of claim 19, wherein said cells are human.

26. The stem cells of claim 19, wherein said cells are non-human animal cells selected from the group consisting of dog, cat, mouse, rat, cow, pig, sheep, goat, horse, buffalo, llama, ferret, guinea pig and rabbit.

25 27. The stem cells of claim 19, wherein the nuclear DNA has been genetically modified.

30 28. The stem cells of claim 19, wherein said enucleated ooplastoid comprises less than the cytoplasmic volume of the original egg from which it is derived.

29. The stem cells of claim 19, wherein said enucleated ooplastoid comprises from about 10% to about 100% of the cytoplasmic volume of the original egg from which it is derived.

30. A stem cell which is produced by the method of (i) contacting a desired somatic cell or somatic cell nucleus with an ooplastoid, wherein said ooplastoid is derived from an enucleated oocyte; (ii) combining said somatic cell or somatic cell nucleus with said ooplastoid to create a nascent cell, and (iii) culturing said nascent cell to obtain pluripotent non-embryonic stem cells.

31. The stem cells of claim 30, wherein said cells are negative for expression of the SSEA-1 marker.

32. The stem cells of claim 30, wherein said cells express elevated alkaline phosphatase activity.

33. The stem cells of claim 30, wherein said cells are positive for expression of the TRA-1-81 marker and the TRA-1-60 marker.

34. The stem cells of claim 30, wherein said cells are positive for expression of the CCA-3 and CCA-4 Markers.

35. The stem cells of claim 30, wherein said cells differentiate into cells derived from mesoderm, endoderm and ectoderm germ layers when the cells are injected into a SCID mouse.

36. The stem cells of claim 30, wherein said cells are human.

37. The stem cells of claim 30, wherein said cells are non-human animal cells selected from the group consisting of dog, cat, mouse, rat, cow, pig, sheep, goat, horse, buffalo, llama, ferret, guinea pig and rabbit.

38. The stem cells of claim 30, wherein the nuclear DNA has been genetically modified.

39. The stem cells of claim 30, wherein said enucleated ooplastoid comprises less than the cytoplasmic volume of the original egg from which it is derived.

40. The stem cells of claim 30, wherein said enucleated ooplastoid comprises from about 10% to about 100% of the cytoplasmic volume of the original egg from which it is derived.

41. A nascent cell produced from the combination of a somatic cell nucleus and an enucleated zona pellucida free ooplastoid.

42. The nascent cell of claim 41, which is activated by a series of electrical pulses.

43. The nascent cell of claim 41, which is activated by the addition of a chemical activator.

44. The nascent cell of claim 41, which is activated by the addition of a chemical activator selected from the group consisting of ethanol, inositol trisphosphate, calcium ionophores, strontium ions, 6-dimethylaminopurine, cyclohexamide, and phorbol 12-myristate 13-acetate.

45. The nascent cell of claim 41, having from about 10% to about 100% of the cytoplasmic volume of the original egg from which it is derived.

46. The nascent cell of claim 41, having less than 50% of the cytoplasmic volume of the original egg from which it is derived.

47. A method of producing pluripotent, non-embryonic stem cells comprising the following steps:

(i) contacting a desired somatic cell or somatic cell nucleus with an ooplastoid, wherein said ooplastoid is derived from an enucleated oocyte;

(ii) combining said somatic cell or somatic cell nucleus with said ooplastoid to create a nascent cell;

(iii) activating said nascent cell; and

(iv) culturing said nascent cell to obtain pluripotent non-embryonic stem cells.

48. The method according to claim 47, wherein said somatic cell or somatic cell nucleus is a mature cell.

49. The method according to claim 47, wherein said somatic cell is an epithelial cell, lymphocyte or fibroblast.

50. The method according to claim 47, wherein said combining step involves intracytoplasmic injection of the somatic cell nucleus into the zona free reduced volume ooplastoid.

5 51. The method according to claim 47, wherein said combining step involves fusion in an electric field via electroporation.

52. The method according to claim 47, wherein said combining step involves fusion induced by electrodes that are introduced directly into the culture dish and electrical pulses
10 administered to the couplets immediately following micromanipulation.

53. The method according to claim 47, wherein said combining step involves fusion in a fusion chamber.

15 54. The method according to claim 47, wherein said ooplastoid contains less than 50% of the cytoplasmic volume of a mature oocyte.

55. The method according to claim 47, wherein said ooplastoid contains from about 10% to about 100% of the cytoplasmic volume of a mature oocyte.

20 56. A cell line obtained according to the method of claim 47.

57. A method of producing pluripotent non-embryonic stem cells comprising the following steps:

- 25 (i) contacting one or more desired somatic cells or somatic cell nuclei with a super-ooplast derived from one or more enucleated zona pellucida free oocytes;
(ii) dividing said super-ooplast into single nucleus containing nascent cells;
(iii) activating said nascent cells; and
(iv) culturing said nascent cells to obtain pluripotent non-embryonic stem cells.

30 58. The method according to claim 57, wherein said enucleated zona pellucida free super-ooplast comprises more than 100% of the cytoplasmic volume of a single egg.

35 59. The method according to claim 57, wherein said somatic cell or somatic cell nucleus is a mature cell.

60. The method according to claim 57, wherein said somatic cell is an epithelial cell, lymphocyte or fibroblast.

61. The method according to claim 57, wherein said dividing step involves partitioning said super-ooplast into separate single nuclei containing nascent cells.

62. The method according to claim 57, wherein said contacting step involves intracytoplasmic injection of said somatic cell nucleus into said super-ooplast.

63. The method according to claim 57, wherein said activation step involves fusion in an electric field via electroporation.

64. The method according to claim 57, wherein said activation step involves fusion in a fusion chamber.

65. The method according to claim 57, wherein said activation step involves fusion induced by electrodes that are introduced directly into the culture dish and electrical pulses administered to the couplets immediately following micromanipulation.

66. The method according to claim 57, wherein said nascent cell is activated using electrical pulses.

67. The method according to claim 57, wherein said nascent cell is activated during a fusion process.

68. A cell line obtained according to the method of claim 57.

69. A method of producing an ooplastoid comprising the following steps:

- (i) harvesting an oocyte from a female;
- (ii) maturing said oocyte to metaphase II;
- (iii) breaching or removing the zona pelucida of said metaphase II oocyte;
- (iv) enucleating said oocyte by removing the polar body and nuclear DNA of said oocyte through the breach of the zona pelucida or by oocyte partitioning; and
- (v) aspirating and pinching off an ooplastoid from said enucleated oocyte.

70. The method of claim 69, wherein said oocyte is from a human.

71. The method of claim 69, wherein said oocyte is from a non-human animal selected from the group consisting of dog, cat, mouse, rat, cow, pig, sheep, goat, horse, buffalo, llama, ferret, guinea pig and rabbit.

72. The method of claim 69, wherein said zona pelucida is breached or removed using a chemical agent.

73. The method of claim 69, wherein said zona pelucida is breached or removed using mechanical action.

74. The method of claim 69, wherein said ooplastoid has from about 10% to about 100% of the volume from the original oocyte.

75. The method of claim 69, wherein said ooplastoid has from about 15% to about 49% of the volume from the original oocyte.

76. The method of claim 69, wherein said ooplastoid has from about 17% to about 33% of the volume from the original oocyte.